EXHIBIT D

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A Simple and General Entry to Aplysinopsine- Type Alkaloids by Tandem Aza-Wittig/Heterocumulene-Mediated Annelation.

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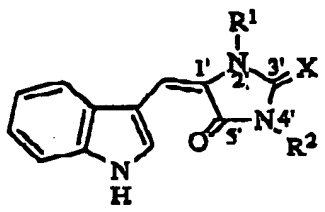
Abstract.- Aza/Wittig-type reactions of iminophosphorane 8, derived from ethyl α-azido-β-(3-indolyl)propenoate and triphenylphosphine, with methyl isocyanate, carbon dioxide or carbon disulfide lead to the corresponding heterocumulenes 9, 10, and 11 which undergo cyclization by the action of nitrogenous reagents to give Aplysinopsine derivatives.

There is considerable interest in Aplysinopsine-type alkaloids which have significant antineoplastic activity[1]. Aplysinopsine itself **1** was first isolated from the sponge genus *Thorecta* of the Australian Great Barrier Reef[2]. A number of related indole alkaloids have also been isolated from both dendrophylliids and sponges. Thus, 2'-demethyl-3'-methylaplysinopsine **2** and 3'-deimino-3'-oxoaplysinopsine **3** have been isolated from dendrophylliid coral *Tubastraea* sp. collected at Palawan, Philippines[3,4], and the 3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsine **4** isolated from the Mediterranean dendrophyllids *Leptopsammia pruvoti*[4].



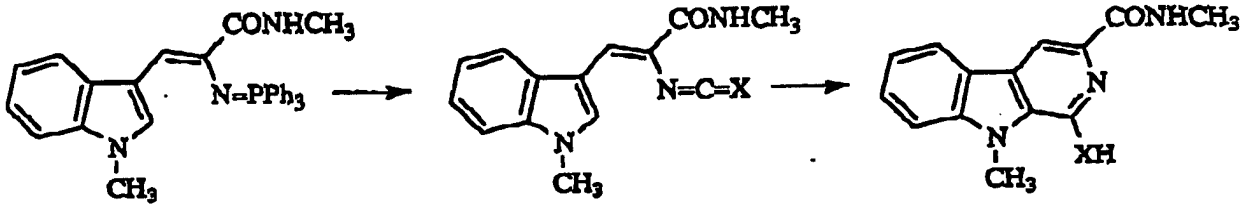
1	R^1= Me	R^2= Me	X= NH
2	R^1= H	R^2= Me	X= NMe
3	R^1= Me	R^2= Me	X= O
4	R^1= H	R^2= H	X= O

Synthetic approaches towards Aplysinopsine-type structures involve base-catalyzed condensation of the appropriate 3-formylindole with a five-membered ring α-methylene carbonyl compound[3-6].

In the course of our studies directed towards the synthesis of fused heterocycles based on the heterocyclization reaction of C=C-conjugated heterocumulenes, we have developed two differents approaches to the synthesis of nitrogen heterocycles: the so-called tandem aza-Wittig/ electrocyclic ring closure[7] and tandem aza-Wittig/ heterocumulene-mediated annelation[8]. Recently, the former has been used for the construction of the framework of the alkaloids Lavendamycin and Eudistomins[9], we now report a slight modification of the latter strategy, underkaten in order to prepare some imidazole derivatives of valuable biological interest. Our approach is centered on the aza Wittig-type reaction of the iminophosphorane **8** derived from ethyl α-azido-β-(3-indolyl)propenoate with methyl isocyanate, carbon dioxide and carbon disulfide to give unsaturated heterocumulenes which undergo cyclization to give the imidazole ring.

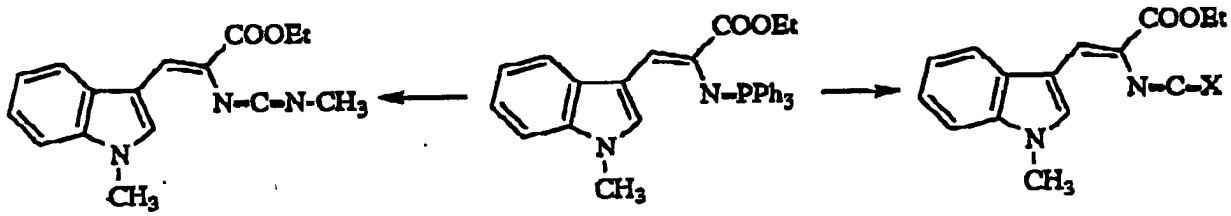
At first, we have tried to prepare the Aplysinopsine framework from the iminophosphorane **5**, available from

3-formylindole by standard chemistry: condensation with ethyl azidoacetate, hydrolysis, amidation with methylamine and further Staudinger reaction with triphenylphosphine. Reaction of iminophosphorane **5** with methyl isocyanate, carbon dioxide and carbon disulfide afforded the corresponding heterocumulenes **6** (X=NCH_3;O;S) which by heating undergo electrocyclic ring-closure to give β-carbolines **7** and no products derived from the nucleophilic attack of the amide group on the heterocumulene moiety were observed.



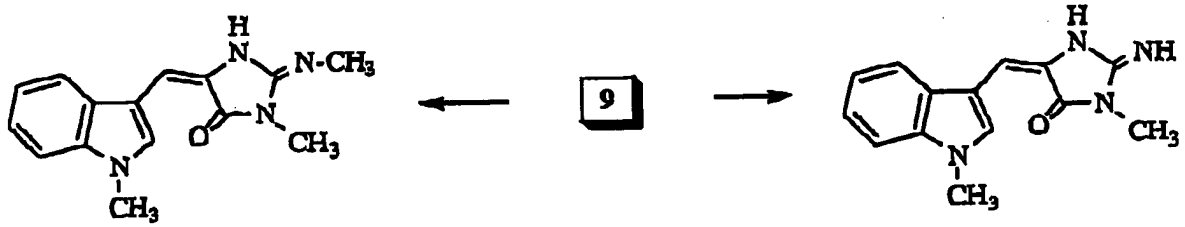
5 **6** **7**

The formation of the imidazole ring of the Aplysinopsine is achieved from iminophosphorane **8**, easily prepared from 3-formyl-1-methylindole[10], via heterocumulenes **9-11**. Compound **8** reacts with methyl isocyanate at room temperature to give the carbodiimide **9** in 85% yield. Likewise, the reaction with carbon dioxide and carbon disulfide in dry toluene at 90°C affords the isocyanate **10** (80%) and the isothiocyanate[10] **11** (89%), respectively.



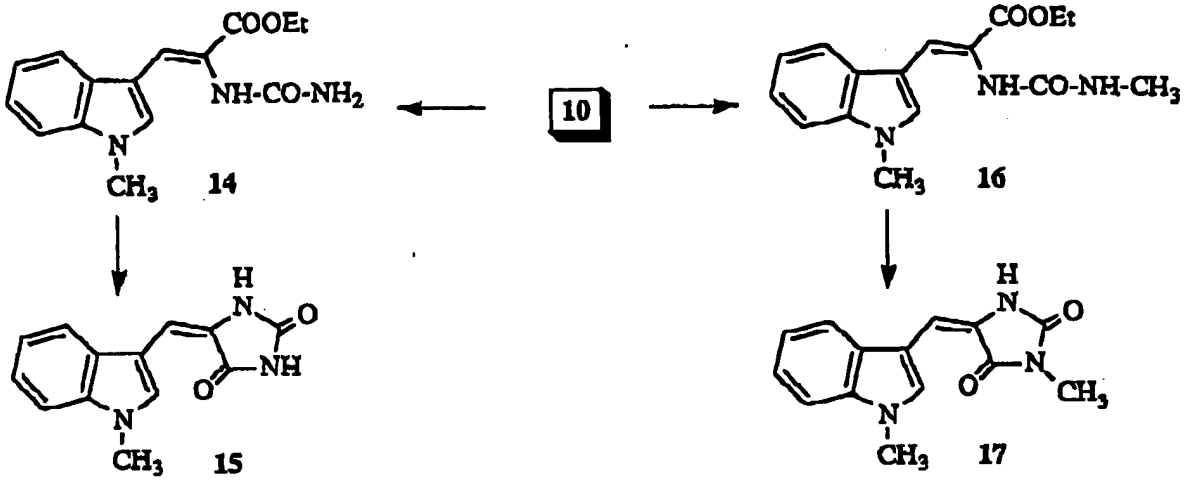
9 **8** **10** X=O **11** X=S

Carbodiimide **9** reacts with ammonium acetate in acetonitrile at 45°C to give **12** in 40% yield[11] thus completing the assemblage of the carbon skeleton of Aplysinopsine through a guanidine-substituted intermediate which undergoes intramolecular cyclization across the ester functionality. Conversion of the carbodiimide **9** into 2'-demethyl-3'-methylaplysinopsine[12] **13** is achieved in 80% yield by reaction with methylamine in toluene at 45°C.

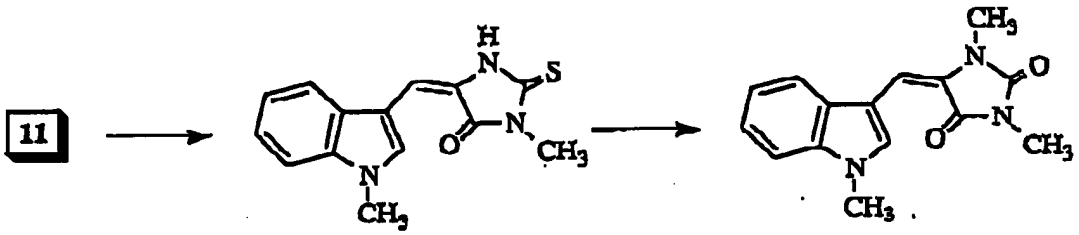


13 **12**

The isocyanate **10** reacts with ammonium acetate in acetonitrile at room temperature to give the urea **14** (78%) which undergoes cyclization by the action of acetic anhydride to give the 3'-deimino-2',4'-bis(demethyl)-3'-oxo aplysinopsine **15** in 50% yield[13]. Similarly, compound **10** by reaction with methylamine and further cyclization by the action of acetic anhydride leads to **17** in 50% overall yield.



Finally, isothiocyanate **11** reacts with methylamine in toluene at 45°C directly giving **18** in 90% yield, which by sequential treatment with dimethyl sulfate and further alkaline hydrolysis affords the 3'-deimino-3'-oxoaplysinopsine **19** in 78% yield[14].



18 **19**

In conclusion the results reported here show that the tandem aza-Wittig/heterocumulene-mediated strategy affords a new and versatile entry to a variety of Aplysinopsine derivatives[15] bearing a nitrogen, oxygen or sulfur atom at position 3', in the imidazole ring. Taking into account that the main structural difference between the natural ocurring Aplysinopsine- type alkaloids is found to be the type of functionalization at the imidazole ring, the method described here shows to be a useful alternative to those previously reported which need as precursors appropiate functionalized imidazole derivatives to construct the Aplysinopsine framework.

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11. *Typical Procedure*: To a solution of iminophosphorane **8** (0.5g, 1mmol) in dry toluene (50ml)was added dropwise a solution of methyl isocyanate (0.057g, 1mmol) in the same solvent (10ml) at room temperature under nitrogen. The reaction mixture was stirred at this temperature for 35 h. The solvent was removed under reduced pressure and the crude carbodiimide **9** was used without purification. To a solution of carbodiimide **9** (0.28g, 1mmol) in dry acetonitrile (50ml) was added in one portion ammonium acetate (0.15g, 2mmol). The mixture was heated at 45°C for 14 h. after cooling the precipitated solid was collected by filtration, washed with water (2x10ml), air-dried and recrystallized from toluene to give **12** (0.1g, 40%) as brown crystals, m.p. 169-71°C. ^{1}H n.m.r. (300 MHz, DMSO-d_6) δ 3.06 (s, 3H, CH_3-N_a), 3.81 (s, 3H, CH_3-N), 4.35 (br, 2H, NH), 6.76 (s, 1H, H-8),7.11 (td, 1H, ^{3}J=7.9Hz,^{4}J=1.1Hz, H-5), 7.20 (td, 1H, ^{3}J= 8.2Hz, ^{4}J=1.1Hz, H-6), 7.4(d,1H ^{3}J= 8.2Hz, H-7), 7.91 (d, 1H, ^{3}J= 7.9Hz, H-4), 8.13 (s, 1H, H-2); ^{13}C n.m.r. (75 MHz, DMSO-d_6) δ 25.50 (CH_3-N_a), 32.77 (CH_3-N), 106.47 (C-8), 109.91 (C-7), 110.98 (C-3), 118.95 (C-4), 119.83 (C-5),121.93 (C6), 127.30 (C-3a), 132.51 (C-2), 136.39 (C-1'), 136.63 (C-7a), 157.51 (C-3'), 169.09 (C-5'); m/z (%) 254 (M^+,90).
12. Compound **13**. ^{1}H n.m.r. (300 MHz, DMSO-d_6) δ 3.01 (d, 3H, ^{3}J= 4.3Hz, CH_3-NH), 3.04 (s, 3H, CH_3-N_a), 3.84 (s, 3H, CH_3-N), 6.78 (s, 1H, H-8), 7.12 (t, 1H, ^{3}J= 7.5 Hz, H-5), 7.20 (t, 1H, ^{3}J= 7.9Hz, H-6), 7.40 (d, 1H, ^{3}J= 4.3Hz, NH), 7.45 (d, 1H, ^{3}J= 7.9Hz, H-7), 7.96 (d, 1H, ^{3}J=7.5Hz, H-4), 8.26 (s, 1H, H-2); ^{13}C n.m.r. (75 MHz, DMSO-d_6) d 25.37 (CH_3-NH), 27.81 (CH_3-N_a), 32.89 (CH_3-N), 106.68 (C-8), 110.04 (C-7), 110.83 (C-3), 118.93 (C-4), 119.85 (C-5), 121.88 (C-6), 127.20 (C-3a), 132.82 (C-2), 136.09 (C-1'), 136.43 (C-7a), 156.90 (C-3'), 169.03 (C-5'); m/z (%) 268 (M^+, 13).
13. Compound **15**. ^{1}H n.m.r. (300 MHz, DMSO-d_6) δ 3.82 (s, 3H), 6.71 (s, 1H, H-8), 7.17 (td, 1H, ^{3}J= 8.1Hz, ^{4}J= 1.2Hz, H-5), 7.24 (td, 1H, ^{3}J= 8.1Hz, ^{4}J= 0.9Hz, H-6), 7.49 (d, 1H, ^{3}J= 8.4Hz, H-7), 7.76 (d, 1H, ^{3}J= 7.7Hz, H-4), 8.12(s, 1H, H-2), 10.18 (br, 2H, NH); ^{13}C n.m.r. (300 MHz, DMSO-d_6) δ 33.01 (CH_3-N), 101.25 (C-8), 107.52 (C-3), 110.25 (C-7), 118.21 (C-4), 120.35 (C-5), 122.38 (C-6), 123.69 (C-3a), 127.25 (C-1'), 130.76 (C-2), 136.33 (C-7a), 155.28 (C-3'), 165.33 (C-5'); m/z (%) 241 (M^+, 48).
14. Compound **19**. ^{1}H n.m.r. (300 MHz, DMSO-d_6) δ 2.87 (s, 3H, CH_3-N_a), 3.79 (s, 6H, CH_3-N /CH_3-N_2), 6.52 (s, 1H, H-8), 7.09 (t, 1H, ^{3}J= 7.7 Hz, H-5), 7.18 (t, 1H, ^{3}J= 7.3Hz, H-6), 7.42 (d, 1H, ^{3}J= 8.1Hz, H-7), 7.74 (d, 1H, ^{3}J= 7.7Hz, H-4), 8.13 (s, 1H, H-2); ^{13}C n.m.r. (75 MHz, DMSO-d_6) δ 24.17 (CH_3-N_a), 32.80 (CH_3-N), 52.91 (CH_3-N_2), 98.93 (C-8), 109.75 (C-3), 109.94 (C-7), 118.31 (C-4), 119.65 (C-5), 121.82 (C-6), 127.25 (C-3a), 130.71 (C-2), 131.50 (C-1'), 136.22 (C-7a), 158.82 (C-3'), 1676.04 (C-5'); m/z (%) 269 (M^+, 11).
15. Satisfactory ^{1}H, ^{13}C n.m.r. (values assigned by decoupling methods and 2D ^{1}H-^{13}C correlation techniques), mass spectra and elemental analyses were obtained for all new compounds.

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